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SPECIAL TOPIC: Translational medicine in China I
• In Memoriam: Professor Liu Shih-Hao •

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Liu Shih-Hao: Pioneer of translational medicine in China

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Editorial comment Translational medicine is a new discipline which aims to eliminate the barrier between preclinical and clinical medicine. Here, Dr. Li discusses the application of translational medicine in the research, teaching and clinical work of Prof. Liu Shih-Hao, the founder of endocrinology in China, who was particularly renowned for his early work in calcium and phosphorus metabolism. This well-known success story can be traced back to an early appreciation of translational medicine by Prof. Liu Shih-Hao, and serves as an important and revelatory lesson for us all.

translational medicine, Liu Shih-Hao

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Translational medicine as a unique discipline formally evolved from the translational research of the last century. Its objective is to apply the results of preclinical medicine directly to clinical medicine, and also to identify challenges in clinical medicine for further mechanistic studies in the laboratory in order to develop new methods to address these clinical problems. In a short phrase, the essence of translational research is "Bench to Bedside and Bedside to Bench" [1]. Although translational research has only relatively recently been formally declared a new discipline in the first issue of the *Journal of Translational Medicine* in 2003, the principles behind translational medicine have existed much longer. By delving into the history of medicine from the first half of the 20th century, it is clear that Prof. Liu Shih-Hao (刘士豪), the founder of endocrinology in China, did develop his research, teaching and clinical work according to these fundamental principles of translational medicine.

1 Brief introduction of Dr. Liu Shih-Hao

Liu Shih-Hao (1900–1974) (Figures 1 and 2) was born in Wuchang County, Hubei Province of China in 1900. He entered Boone Middle School, which was a missionary school, in 1913 where he completed six years of courses in only four years. He joined the Premedical School of Hunan-Yale Medical College in 1917, and transferred to the Premedical School of Peking Union Medical College (PUMC) in 1919 and the Medical School in 1920. He graduated in 1925, receiving a Medical Doctor degree from New York State University, and the Wenham Prize for having the highest total score.

In his last year as a medical student at PUMC, Liu Shih-Hao wrote his first paper entitled "The Influence of Cod Liver Oil on the Calcium and Phosphorus Metabolism in Tetany" [2], which was published in the *China Medical Journal*. This paper reflected his strong interest in endocrinology, and the Department of Internal Medicine of PUMC Hospital naturally accepted him after graduation. Liu was the first graduate from PUMC to work in this department.

From 1925 to 1928 Liu served as intern, resident, chief resident and attending physician. He published 13 papers during that period, on topics ranging from hypoglycemia to acid-base balance of malaria, which laid a solid foundation for his clinical research.

From 1928 to 1930, Liu worked at the Rockefeller Institute of Medicine as a visiting scholar, where he studied

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metabolic disease and blood gas analytical techniques under renowned biochemist D. D. Van Slyke. He and his colleagues established a method for determining pH, the concentration of CO and CO_2 tension in blood samples, and a new method for research into the acid-base balance of blood.

After returning to China in 1930, Liu focused his research on calcium and phosphorus metabolism. He became the leader of the research group on this discipline after R. R. Hannon returned to the United States in 1934. He and other members of this group, including Chu Hsieh-I, Wang Shu-Hsien, Chou Shou-Kai and Yu Tsai-Fan, published a series of papers on the bone disease osteomalacia. These 13 papers, collectively entitled "Calcium and Phosphorus Metabolism of Osteomalacia" [3–15] described many features of osteomalacia, from the influence of food to the function of vitamin D, and made a unique move into the realm of bone metabolism, marking an important discovery for international endocrinology. A. Parfitt, the well-known endocrinologist, highly appraised Liu and his colleagues' research in a special article commemorating Prof. Chu Hsien-I [16], "for many years the PUMC papers constituted the world's entire stock of knowledge concerning the metabolic aspects of human privational vitamin D deficiency and its treatment".

One of the more outstanding achievements of Liu's research on calcium and phosphorus metabolism during this period was the nomination of the disease "renal osteodystrophy". In April 1942, Liu Shih-Hao and Chu Hsien-I published an article [17] in the journal *Science* proposing the name "renal osteodystrophy" and the effectiveness of treatment by dihydrotachysterol (A.T.10). Different from the traditional nominations of syndromes based on phenomenology, the name "renal osteodystrophy" reflects the nature of the disease, which has assured its wide-spread adoption that continues to this day.

During the same period, Liu Shih-Hao diagnosed and treated the first Chinese case of insulinoma, and performed some detailed metabolic research with the cooperation of Loucks, who was the head of the Department of Surgery. The paper reporting their findings [18] was published in the prestigious *Journal of Clinical Investigation*.

From 1938 to 1939, Liu Shih-Hao was in London, and systematically performed animal experiments under Edward Charles Dodds, the famous scholar of the Courtauld Institute of Biochemistry at Middlesex Hospital. With the cooperation of R. L. Noble, he stimulated the hypophysectomized Wistar rats with pregnant mare serum and human pregnancy urine, and observed the resultant changes in the male/female reproductive system, thus paving the way for endocrine research in laboratory animals [19,20].

In 1941, Liu Shih-Hao became the first professor in PUMC among PUMC graduates. However, he was forced to practice medicine at the end of that year because PUMC was occupied by the Japanese army. When the Second World War ended, Liu Shih-Hao was invited to be the Chief of Internal Medicine at Tongren Hospital, a special doctor at the Peking Army Hospital, and in 1948 he became the President of Tongren Hospital. After the rehabilitation of PUMC, he went back to work at the PUMC Hospital. After the Korean War broke out, Liu Shih-Hao became head of the Biochemistry Department at the Medical College, while still an internal professor at the PUMC Hospital and President of Tongren Hospital. While working in the Biochemistry Department, he spent a lot of time developing a method for testing hormones. During that time, his book entitled *The Connection Between Biochemistry and Clinical Medicine* (in Chinese) [21], was published, which has influenced a generation of doctors working in internal medicine.

In 1958, Liu Shih-Hao quit his jobs in the Biochemistry Department and Tongren Hospital, and built an endocrinology department at the PUMC Hospital, becoming its first Chief. At the beginning of the 1960s, Prof. Liu Shih-Hao observantly noted the potential importance of the invention of radioimmunoassays for endocrinology, and decided to develop the method immediately, which he completed in 1965. At the same time, he led the survey of diabetes mellitus incidence in workers from the Capital Iron and Steel Works, and built a solid foundation for the following epidemiology survey and research on diabetes mellitus in our country. Besides all of this, he opened four senior seminars

Figure 1 Liu Shih-Hao was doing the experiment.

Figure 2 Liu Shih-Hao and his wife Wang E-Tsung (1957).

at the beginning of the 1960s, which trained teachers and researchers throughout the country.

In June 1974, Prof. Liu Shih-Hao, impacted by the Cultural Revolution, was wronged and driven to death.

Prof. Liu Shih-Hao was the founder and pioneer of endocrinology in China, and his acknowledgement of the principles behind translational medicine helped him to achieve these significant contributions to science and medicine.

2 The translational medical aspect of Liu Shih-Hao's research on calcium and phosphorus metabolism

In Prof. Liu Shih-Hao's article on calcium and phosphorus metabolism, we have already seen the principles of "Bench to Bedside and Bedside to Bench", even though the concept of translational medicine had not been formally defined.

In 1930's China, one of the main clinical problems was the high morbidity of osteomalacia, particularly in the north. Osteomalacia is a disease caused by defective bone mineralization which manifests as bone ache, skeleton deformity and bone fractures. Complications include delivery failure caused by deformity of the pelvis and tetany of the neuromusculature. The most severely affected patients become disabled and unable to take care of themselves. It was believed that lack of sunshine in northern China was the cause of osteomalacia. The Chief of the Department of Gynecology and Obstetrics at PUMC, J. P. Maxwell, published an article [22] entitled "Osteomalacia in China", which discussed this problem in detail. When Liu Shih-Hao began to study calcium and phosphorus metabolism, the vitamin D deficient animal model [23] had been established, and was treatable with vitamin D. The aim of translational medicine is to convert insight gained from preclinical medicine to therapeutic options for the clinic. Liu Shih-Hao and his research group published serial articles entitled "Calcium and Phosphorus Metabolism in Osteomalacia" [3–15]. In the 13 papers, they discussed in depth the treatment and prevention of osteomalacia, resulting in effective treatments for osteomalacia, which was particularly important for pregnant and nursing women.

By investigating the specific content of this work, we can identify research topics that fit in with modern translational medicine. The subtitles of these 13 papers are

(i) The effect of vitamin D and its apparent duration;

(ii) Further studies on the response to vitamin D of patients with osteomalacia;

(iii) The effects of varying levels and ratios of intake of calcium to phosphorus on their serum levels, paths of excretion and balances;

(iv) Report of an unusual case in a male with acute parathormone poisoning;

(v) The effect of varying levels and ratios of calcium to phosphorus intake on their serum levels, paths of excretion and balances in the presence of continuous vitamin D therapy;

(vi) The added drain of lactation and beneficial action of vitamin D;

(vii) The effect of ultraviolet irradiation from mercury vapor quartz lamp and sunlight;

(viii) The effects of ingestion of acid and alkali in patients with and without chronic nephritis;

(ix) Metabolic behavior of infants fed on breast milk from mothers showing various states of vitamin D nutrition;

(x) Further studies on vitamin D action: Early signs of depletion and effect of minimal doses;

(xi) The pathogenetic role of pregnancy and relative importance of calcium and vitamin D supply;

(xii) Studies of calcium and phosphorus metabolism with special reference to pathogenesis and effect of dihydrotachysterol (A.T. 10) and iron;

(xiii) The availability of inorganic phytin, and dietary phosphorus and the effect of vitamin D.

The main research area of Liu Shih-Hao *et al.* in the Metabolism Ward of the PUMC Hospital was the detailed metabolic analysis of osteomalacia patients. The team accurately measured the quantities of calcium and phosphorus intake from food and drinks, and excretion in urine and stool, in these patients. The meals were prepared in duplicate so one portion could be analyzed in the laboratory for levels of calcium and phosphorus. After a period of 4 d, serum calcium and phosphorus levels were measured for each patient to monitor changes. By observing the metabolism of calcium and phosphorous during different periods of food and drug treatments, they could make conclusions regarding the underlying pathophysiological mechanisms in order to develop the best therapeutic regimen. For example, in "Calcium and Phosphorus in Osteomalacia (V): The Effect of Varying Levels and Ratios of Calcium to Phosphorus Intake on Their Serum Levels, Paths of Excretion and Balances in the Presence of Continuous Vitamin D Therapy" [7] published in the *Journal of Clinical Investigation* in 1937, an osteomalacia patient receiving continuous vitamin D therapy was analyzed for changes in calcium and phosphorous metabolism when eating different foods. Figure 1 shows these changes in calcium and phosphorus levels: The solid line represents serum calcium levels; the dashed line represents the intake of calcium and phosphorus levels; the open bars indicate the quantity of calcium and phosphorus in stool; the shaded bars indicate the excretion of calcium and phosphorus in urine. There were 12 types of food given to these patients, containing three different amounts of calcium and four different amounts of phosphorus. The patients ate each type of food for three defined metabolic periods. Serum calcium and phosphorus levels were analyzed in the morning at the beginning of each period, and the urine and stool excreted during each period were also tested for calcium and phosphorus levels. Figure 1 shows that the

serum calcium levels were quite stable, but the serum phosphorus level changed dramatically. For example, in the 15th period, serum phosphorus levels rose by 5.3 mg dL^{-1}, and, while the calcium intake remained at a high level, phosphorus intake gradually increased with no calcium tested in urine, and phosphorus excretion increased in urine but kept steady in stool. Liu Shih-Hao analyzed each metabolic period in order to make conclusions regarding calcium and phosphorus metabolic balance. At the same time, they performed an analogous study in syphilis ostitis patients (who were not taking vitamin D), who displayed no obvious metabolic changes and were used as a control. The major important conclusion from this work was that when using vitamin D as a preclinical treatment, a 2:1 ratio of calcium and phosphorus in food was most effective for deposition in bone. Thus, given the low levels of calcium in Chinese food, calcium supplementation was required. Liu Shih-Hao and his research group made many more useful conclusions from these studies, converting this preclinical medical study into practical therapeutic approaches for clinical medicine, thus providing clear examples of translational medicine.

The National Institute of Health (NIH) defines two stages in translational medicine: The first stage is confirming laboratory findings in animals and humans; the second is moving these results into clinical practice, in order to find the best therapy for the clinic. The research study of Liu Shih-Hao belonged to the second stage of translational medical research, as he investigated the importance of food composition for patients receiving vitamin D therapy in order to promote the best possible recovery. In addition, the analysis of serum calcium and phosphorus level and their quantities in stool and urine is more of a basic laboratory study. In the 1930s, at the PUMC Hospital, the metabolic research of Liu Shih-Hao was required to combine preclinical and clinical medicine. Thus, "Bench to Bedside and Bedside to Bench" was not only a concept, but also a reality. In addition, the preclinical study of Prof. Wu Hsien on nutriology in the Biochemistry Department, demonstrated the

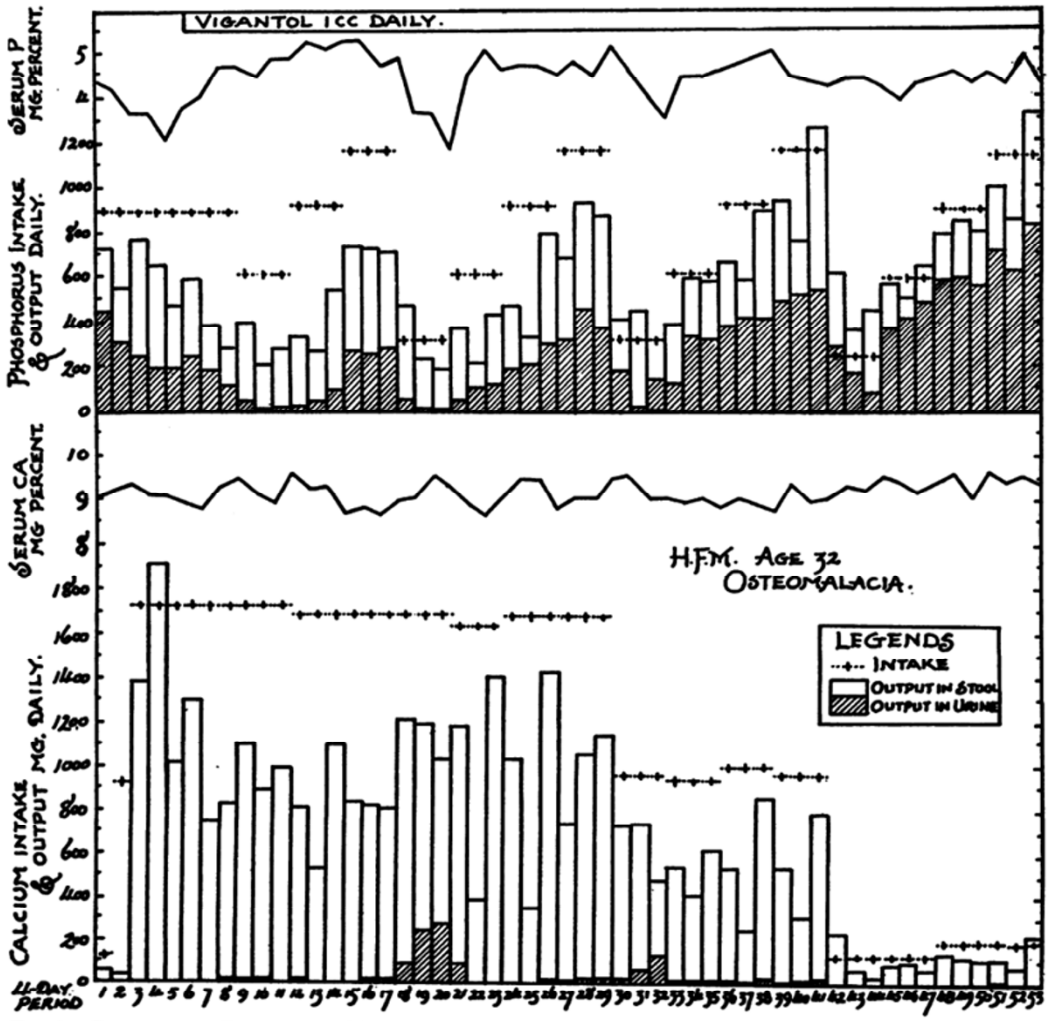

FIG. 1. CALCIUM AND PHOSPHORUS METABOLISM AND THEIR SERUM LEVELS IN RELATION TO VARYING INTAKE OF CALCIUM AND PHOSPHORUS IN SUBJECT 1

Figure 3 Calcium and phosphorus metabolism and their serum levels in relation to varying intake of calcium and phosphorus in ref. [7].

calcium and phosphorus quantities in common foods, which also helped Liu Shih-Hao make his final conclusions and illustrates the essence of translational medicine.

The most outstanding contribution in the field of bone metabolism was "renal osteodystrophy" which was named by Liu Shih-Hao and Chu Hsieh-I, and successfully treated with A.T.10. The success of this work again comes from the consideration and practice of translational medicine. Liu Shih-Hao and his research group performed a metabolic study on chronic renal failure patients accompanied by osteomalacia [24], and found that the essence of this disease was a secondary bone mineralization disorder caused by chronic renal failure and a calcium and phosphorus metabolic disorder. Therefore, they unified the present nomenclature and introduced the disease as "renal osteodystrophy". Because of the simplicity and accuracy of this name, it was widely accepted by academic circles and is still used now. A.T.10 was another medicine that could be used to treat nutritional osteomalacia. The mechanism is essentially the same as vitamin D, but for renal osteodystrophy patients treatment with vitamin D is highly problematic, thus an alternative option was required. Liu Shih-Hao and Chu Hsieh-I treated these patients with A.T.10 and recorded their metabolic profiles to reveal that a regular dose of A.T.10 was an effective therapy for renal osteodystrophy. In addition, they made a further discovery within the realms of translational medicine, specifically in clinical practice. As A.T.10 was effective in renal osteodystrophy while vitamin D was not, they hypothesized that chronic renal failure could affect the activity of vitamin D but not A.T.10. This raised questions about vitamin D and kidney physiology, which, while not answered at the time, was investigated some thirty years later by the American chemist Deluca [25], who also identified the active form of vitamin D *in vitro*, which is 1,25-$(OH)_2$VitD. This discovery led to the industrial production of 1,25-$(OH)_2$VitD and its use as a common therapeutic medicine to treat osteomalacia and renal osteodystrophy in the clinic, illustrating once again the successful application of "Bedside to Bench and Bench to Bedside".

3 The reflection of translational medicine in other work of Liu Shih-Hao

In 1934, Liu Shih-Hao successfully diagnosed and treated the first Chinese case of insulinoma in the Metabolism Department at PUMC [18]. After surgically removing the tumor from the patient, Liu injected tumor extract into a rabbit, and analyzed its insulin content by measuring the decrease in blood glucose levels. Thus, he performed an insulin bioassay and diagnosed insulinoma in an experimental animal, thereby practicing an alternative form of translational medicine.

During 1938 when Liu Shih-Hao was trained in London, he spent most of his time studying experimental animal research. Together with his colleagues, he stimulated Wistar rats, whose pituitary glands had been removed, with pregnant horse serum and pregnant women's urine, which has developed into a method to facilitate ovulation and spermatogenesis using human chorionic gonadotrophin.

In 1951, Liu Shih-Hao became Head of the Department of Biochemistry at PUMC, internal professor at PUMC Hospital and President of Beijing Tongren Hospital, which illustrates his expertise in both the preclinical and clinical arenas.

In 1957, *The Connection Between Biochemistry and Clinical Medicine* (in Chinese) written by Liu Shih-Hao was published [20]. It discussed calcium and phosphorus metabolism and osteopathy, sodium and potassium metabolism, diabetes mellitus, and endocrinal diseases in thyroid, pituitary and adrenal glands from a biochemical and physiological perspective. For many years, every internal doctor working in hospitals across Peking carried a copy of this book. Unfortunately, the plan to reprint it was aborted because of the Cultural Revolution.

In 1958, Liu Shih-Hao combined his research group in the Department of Biochemistry at PUMC with the internal endocrine group at PUMC Hospital, founding the Endocrine Department, where he became Head of Department. This amalgamation further cemented the relationship between preclinical and clinical medicine, facilitating the practice of translational medicine.

During his time as Chief of the PUMC Biochemistry Department, the main focus of Liu Shih-Hao's research was establishing a method to detect hormone, which was a common problem in the clinic. Most endocrine diseases are hyperhormonism or hypohormonism, but at that time the diseases could only be diagnosed by manifestations caused

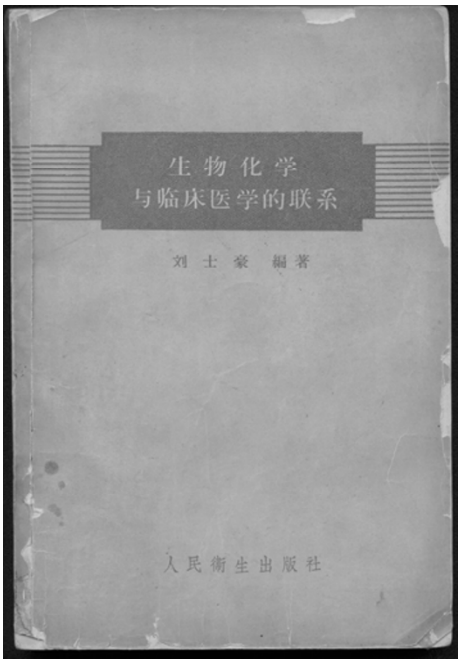

Figure 4 *The Connection Between Biochemistry and Clinical Medicine* (in Chinese).

by the disorder of hormone and were influenced by subjectivity. Thus, being able to precisely detect hormone in blood (or urine) would significantly improve the diagnosis of endocrine diseases. The method for detecting 24-hour urine 17-hydroxycorticosteroids was established by Liu Shih-Hao in 1957, which improved the diagnosis of adrenal diseases. In 1960, American scientists Yalow and Berson established a radioimmunoassay to detect insulin, which improved the precision of hormone detection by several orders of magnitude, and was hailed as a milestone in the history of the endocrinal medical field [26]. When Liu Shih-Hao learned of this progress in China, he realized its significance and began to study the method himself, finishing its setup in 1965. He also planned to detect other hormones by radioimmunoassay, but by then the Cultural Revolution meant he could not continue this work. After the Cultural Revolution, the third Chief of the Endocrine Department, Prof. Shi Yi-Fan, finished the work in the 1980s, which further revised the level of clinical diagnosis for endocrinal diseases. Therefore, the translational medicine approach of Liu Shih-Hao has had a deep influence on Chinese endocrinology.

4 The history behind Liu Shih-Hao's translational approach to medicine

4.1 The translational approach of Liu Shih-Hao was likely influenced by the international reformation of medical education

In 1910, the Flexner report published in the USA recommended an overhaul of American medical education. The report used Johns Hopkins University School of Medicine as an ideal foundation for reforming mainstream medical education. As a result, it closed the gap between biology, preclinical medicine and clinical medicine. This trend of thought in American medical education was highly advanced, helping to make them world leaders in biology and medicine. The Peking Union Medical College (PUMC) is a private medical college founded by the Rockefeller financial group from the USA. The aim was to establish PUMC as a "Johns Hopkins School of Medicine" in the Far East. Liu Shih-Hao was the second graduate of PUMC and deeply influenced by this connection, as well as having studied at the Rockefeller Institute from 1928 to 1930, which helped him to develop a translational approach to his work.

4.2 Liu Shih-Hao had high scholarly achievements in preclinical and clinical medicine

At PUMC, Liu Shih-Hao mainly studied clinical medicine, while also in contact with preclinical medicine professors such as Wu Hsien and Robert KhoSeng Lim. Liu Shih-Hao graduated first in his class from PUMC and worked in the PUMC Hospital for three years in a relatively preclinical area, from which he gained substantial clinical experience. He joined biochemist D. D. Van Slyke at the Rockefeller Institute, enriching his knowledge of biochemistry and developing experimental techniques. He gained a lot from this experience, which was undoubtedly very valuable for his research on calcium and phosphorus metabolism when he returned to China. In 1938, Liu Shih-Hao learned experimental animal techniques in London, which was very advanced fundamental research at that time and also important for translational medicine. Therefore, Liu Shih-Hao's remarkable achievements both in preclinical and clinical medicine set the stage for his work in translational medicine.

4.3 Liu Shih-Hao's pursuit of translational medicine was aided by his working environment

The main reason that Liu Shih-Hao could successfully perform translational medicine was the privileged environment at PUMC. The best example of this was the establishment of renal osteodystrophy and its treatment. The Metabolism Ward in which Liu Shih-Hao worked took as a foundation the world renowned Ward IV at Massachusetts General Hospital, which is affiliated with Harvard Medical School in the USA, of which Fuller Albright made many important discoveries within the field of translational medicine.

4.4 The work of Liu Shih-Hao's predecessors provided the foundation for his research in translational medicine

As mentioned above, the work of Maxwell in epidemiology revealed the importance of osteomalacia in China. Biochemist Wu Hsien was Head of the Biochemistry Department at PUMC, and his research on Chinese diet led to the work of Liu Shih-Hao on metabolism, and is also an important example of translational medicine. At the same time, vitamin D was discovered. Thus, in the 1930s the conditions for the study of osteomalacia matured, and the best place to do this work was at PUMC.

4.5 The divide between preclinical and clinical medicine was not that great

Neither the complexity nor the study scope of the preclinical medicine research in the early 20th century could compare with the current condition. Consequently, during the time of Liu Shih-Hao's career, it was possible to be both a preclinical and clinical expert and study translational medicine. Liu Shih-Hao was Chief of the Biochemistry Department in preclinical medicine and then became Chief of the Endocrine Department in clinical medicine. This combination of position has never recurred in China. However, recently an emphasis has been placed on collaborations between experts from preclinical and clinical areas, highlighting its value.

5 Conclusion

The ideology of Prof. Liu Shih-Hao within his research, clinical work and teaching, which he followed for a lifetime is identical to the current popular trend towards translational medicine. The renowned success attained by Prof. Liu Shih-Hao in translational medicine is both important and revelatory for all of us.

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